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• 計畫中文名稱	麩胺酸受體對胚胎幹原細胞神經性分化之調控功能研究(II)
• 計畫英文名稱	Glutamate Receptor-mediated Neuronal Differentiation in Embryonic Stem Cells (II)
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• 中文關鍵字	麩胺酸鹽; 神經生長因子; 海人酸; P19 細胞; 低氧; 鈣離子; 生長相關蛋白
• 英文關鍵字	Glutamate; Nerve growth factor; Kainic acid; P19 cell; Hypoxia; Calcium ion; Growth associated protein
• 中文摘要	本計畫主要爲使用 P19 細胞株類似幹原細胞的特性，將其分化爲神經元，以研究麩胺酸受體在神經分化過程的功能。首先，我們成功地將 P19 cells 分化成含 85%以上神經元的培養。再者，結果發現離子通道型麩胺酸受體次單元 GluR5,6,7 及 GluR2,3 的表現分別在分化前及分化後 3 天出現，顯示不同亞型的麩胺酸受體在不同階段的神經發育可能扮演重要的角色。以三種離子通道型麩胺酸受體催動劑 NMDA,AMPA, and 紅藻胺酸 K A 均可引發 P19 神經元的內鈣升高，且可升高磷脂胸 C 所引發之多磷酸纖維糖代謝，而代謝型麩胺酸受體催動劑 Trans-ACPD 則否，顯示離子通道型麩胺酸受體在早期神經發育的活性較代謝型受體爲主要。進而結果顯示，紅藻胺酸可降低 P19 神經元在低壓低氧環境下的死亡率，且 AMPA/KA 受體拮抗劑 CNQX 及磷脂胸 C 抑制劑會促進此環境下的死亡率，顯示 AMPA/KA 受體對神經發育早期存活的重要性。流式細胞儀的研究結果顯示，神經生長因子對 P19 細胞自然凋亡的保護必需要在 AMPA 和 K A 的存在下才有顯著的作用，而 AMPA 和 KA 會顯著升高神經生長因子受體中促進存活的 T r k A 而非促進凋亡的 p75NTR 的表現。最後，AMPA 受體也會降低神經纖維生長所需的蛋白 GAP-43 的磷酸化，顯示離子通道型麩胺酸受體對早期發育神經之存活及神經纖維生長均扮演重要的角色。
• 英文摘要	We cultured a P19 mouse teratocarcinoma cell line and induced its neuronal differentiation to study the function of ionotropic glutamate receptors in early neuronal development. Immunocytochemical studies showed 85% neuronal population at 5 DIV with microtubule-associated protein 2- positive staining. Cells expressing the ？\-amino-3-hydroxy-5-methyl-4-isopropinonate (AMPA) receptor subunit, GluR2/3, and the kainate receptor subunit, GluR5/6/7, were 30% and 50%, respectively. In Western blot analysis, the temporal expression of GluR2/3 began to

appear at 3 DIV, whereas GluR5/6/7 was already expressed in the undifferentiated cells. P19-derived neurons began to respond to glutamate, AMPA, and kainate, but not to the metabotropic glutamate receptor agonist, trans-1-aminocyclopentane-1,3-decarboxylic acid (trans-ACPD), by 5 DIV in terms of increases in intracellular calcium and phospholipase C-mediated poly-phospho- inositide turnover. Furthermore, kainic acid reduced cell death of P19-derived neurons in both atmospheric and hypobaric conditions in a phospholipase C-dependent manner. The AMPA/kainate receptor common antagonist, CNQX, butnot the AMPA recept or antagonist, NBQX, profoundly increased hypobaric insult-induced neurotoxicity. In a flow cytometry study, the nerve growth factor-mediated anti-apoptotic effect was facilitated by AMPA, with an induction of TrkA, but not p75NTR expression. Lastly, phosphorylation o f GAP-43, which is involved in neuritogenesis during neurogenesis, was reduced by AMPA receptor antagonist GYKI 52466. Therefore AMPA and kainate receptors might mediate neurotrophic functions to facilitate neurotrophic factor signaling to protect neurons against hypoxic insult in early neuronal development. (Part of this work is accepted by Journal of Biomedical Science on Oct. 7, 2002., and another part will be submitted to Developmental Biology)